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**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 1467-2020

Replacing SN/T 1467-2004

Technical specifications for gosling plague quarantine

小鹅瘟检疫技术规范

(English Translation)

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Foreword

This document was drafted in accordance with the rules given in GB/T 1.1-2020.

This document replaces SN/T 1467-2004 "Gosling Plague Virus Isolation and Agar Immune Diffusion Test Method".

Compared with SN/T 1467-2004, the main technical changes of this document are as follows except for editorial changes.

-Increase the polymerase chain reaction (PCR) method.

--Revised Appendix A, and added Appendix B, Appendix C and Appendix D.

This document was proposed and managed by the General Administration of Customs of the People's Republic of China.

This document was drafted. Nanjing Customs of the People's Republic of China.

1 Scope

This standard specifies the clinical diagnosis, virus isolation test, agar immunodiffusion test and polymerase chain reaction of gosling plague virus.

(PCR) and other technical requirements.

This standard applies to the entry and exit quarantine of gosling plague virus in poultry and its products.

2 Normative references

The contents of the following documents constitute indispensable clauses of the documents through normative references in the text. Among them, dated quotations Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable Used in this document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Terms and definitions

There are no terms and definitions that need to be defined in this document.

4 Clinical diagnosis

For an introduction to gosling plague, see Appendix A, and for the symptoms and pathological changes of gosling plague, see Appendix B.

5.1 Sample collection

5.1.1

For dead or dying animals, samples of liver, pancreas, spleen, kidney, brain, etc. shall be taken aseptically. For live animals, collect whole blood. For animal products, Collect internal organs and tissues.

5.1.2

Sterile equipment should be used for the collection and packaging of samples, and should be sealed, refrigerated and transported to the laboratory immediately after collection.

5.1.3

The sample is made into 10% to 20% suspension with phosphate buffered saline (PBS) containing antibiotics at pH 7.0 ~ 7.4 (see Appendix C for the preparation method).

The floating liquid was centrifuged at 4 °C 3 000 r/min for 10 min, and the supernatant was filtered and sterilized through a 0.22 µm filter membrane. Samples collected or processed at 2 °C ~ Store at 8 °C for no more than 4 days. For long-term storage, it should be placed in a -70 °C refrigerator, but repeated freezing and thawing should be avoided.

5.2.1 Reagents

- Penicillin (working concentration 10 000 IU/mL);
- Streptomycin (working concentration 10 000 IU/mL);
- 9-day-old ~11-day-old SPF goose embryo.

5.2.2 Main equipment

- Biological safety cabinet;
- Centrifuge;
- Constant temperature incubator;
- Micro adjustable pipette.

5.2.3.1 Goose embryo inoculation

Aspirate the filtered and sterilized sample treatment solution, and inoculate at least 5 SPF goose embryos aged 9 to 11 days through the allantoic cavity, 0.2 mL/piece, Incubate at 35 °C ~37 °C for 4 d to 7 d. Check the growth of goose embryos every day after inoculation.

5.2.3.2 Virus harvest

The dead embryos after 18 h and the surviving goose embryos at the end of the culture were collected and placed in a refrigerator at 4 °C for 4 h to 24 h, and the allantoic fluid was collected aseptically.

5.2.4 Virus identification

Use agar diffusion test or polymerase chain reaction (PCR) for gosling plague virus identification.

5.3.1 Reagents and materials

5.3.1.1

Unless otherwise specified, all reagents are analytical reagents or biochemical reagents.

5.3.1.2

Agar plate (see Appendix C for the preparation method).

5.3.1.3

Gosling plague virus positive serum (provided by the designated unit).

5.3.1.4

Gosling plague virus negative serum (provided by the designated unit).

5.3.1.5

Gosling plague virus standard antigen (provided by the designated unit).

5.3.2 Main equipment

Micro adjustable pipette, alcohol lamp, 37 °C thermostat.

5.3.3.1 Preparation of tested serum

Blood was collected to separate serum according to conventional methods. There should be no hemolysis and no bacterial contamination. Store at 4 °C.

5.3.3.2 Treatment of the yolk of the inspected goose

For the treatment of the tested goose egg yolk, use a syringe (without needle) to draw 20 mL of fresh goose egg yolk into a centrifuge tube, and add the same amount of physiological salt. Shake well with water, add 20 mL of chloroform and shake for 30 minutes, centrifuge at 3 500 r/min for 30 minutes, and take the supernatant (Approximately 20 mL) into a dialysis bag, put in 40% polyethylene glycol and concentrate (approximately 6 h~12 h) to 1 mL~2 mL, aspirate the concentrated solution and use. Wash the dialysis bag with 1 mL ~ 2 mL of normal saline, aspirate and combine in the concentrated solution, and control the final concentration to 5 times. Finally at 3 500 r/min Centrifuge for 30 minutes, add 0.01% thimerosal to the supernatant, and freeze and store (-20°C) for inspection.

5.3.3.3 Punch

The reaction hole is used and punched now. On the prepared agar plate, punch holes according to Figure 1, with a hole diameter of about 5 mm and a hole distance of 2 mm~5 mm. Put in the hole Use a needle to gently pick out or aspirate the agar, do not damage the edge to avoid leakage.

figure 1

5.3.3.4 Back cover

Use an alcohol lamp to lightly bake the bottom of the plate or glass plate, and close the bottom of the hole to prevent side leakage.

5.3.3.5 Dosing

Use a micropipette to suck the antigen suspension and add it to well G, add the positive serum control to the well, and press the test serum or egg yolk supernatant. Add them to the remaining holes according to the order, and each hole should be filled with no overflow. Change a tip for each sample added.